

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A01N 63/00, A61K 37/00, C07H 17/00, C12N 5/00, 7/00, 15/00		A1	(11) International Publication Number: WO 94/23582 (43) International Publication Date: 27 October 1994 (27.10.94)
(21) International Application Number: PCT/US94/03831 (22) International Filing Date: 7 April 1994 (07.04.94) (30) Priority Data: 08/044,406 8 April 1993 (08.04.93) US (60) Parent Application or Grant (63) Related by Continuation US 08/044,406 (CIP) Filed on 8 April 1993 (08.04.93) (71) Applicants (for all designated States except US): GENETIC THERAPY, INC. [US/US]; 938 Clopper Road, Gaithers- burg, MD 20878 (US). UNIVERSITY OF CINCINNATI [US/US]; College of Medicine, Elland and Bethesda Av- enues, Cincinnati, OH 45229-2899 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): TRAPNELL, Bruce [US/US]; 4023 Byrd Road, Kensington, MD 20895 (US). WHITSETT, Jeffrey [US/US]; 5565 Salem Road, Cincinnati, OH 45230 (US).		(74) Agents: OLSTEIN, Elliot, M. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068-1739 (US). (81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	
(54) Title: ADENOVIRAL VECTORS INCLUDING DNA ENCODING LUNG SURFACTANT PROTEIN			
(57) Abstract An adenoviral vector including a DNA sequence encoding a lung surfactant protein. The adenoviral vector may be a replication deficient adenoviral vector which is free of at least the majority of the E1 and E3 DNA sequences. Such vectors may be employed for generation of infectious viral particles which may transduce lung epithelial cells <i>in vivo</i> to enable the expression of lung surfactant protein by such cells.			
Best Available Copy			
ATTORNEY DOCKET NUMBER: 7639-061 SERIAL NUMBER: 08/333,680 REFERENCE: AX			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ADENOVIRAL VECTORS INCLUDING DNA
ENCODING LUNG SURFACTANT PROTEIN

This application is a continuation-in-part of application Serial No. 08/044,406, filed April 8, 1993.

This invention relates to adenoviral vectors. More particularly, this invention relates to adenoviral vectors which include DNA encoding a lung surfactant protein and to the use of such vectors in treating disease states associated with lung surfactant protein deficiency, such as infant respiratory distress syndrome, and adult respiratory distress syndrome.

Surfactant proteins are natural endogenous proteins produced primarily within the alveolar and airway epithelial cells of the normal lung and interact with phospholipids to maintain the patency of the alveolar structures. When pulmonary surfactant protein concentration on the alveolar surface falls below critical levels, surface tension of the liquid-gas interface increases, thereby leading to alveolar collapse, pulmonary ventilation-perfusion mismatch, and hypoxia. In severe cases, this can lead to death. Intermittent administration of exogenous bovine lung surfactant protein has shown partial, but not

complete remission of the pathophysiology of the surfactant deficiency state.

It is an object of the present invention to provide a recombinant expression vehicle for expressing pulmonary surfactant protein.

It is a further object of the present invention to provide an expression vehicle which will enable prolonged expression of pulmonary surfactant protein in the lung in order to correct the clinical surfactant protein deficiency state and its attendant pathophysiologic effects on gas exchange.

The above objects and others should be apparent from the following specification.

In accordance with an aspect of the present invention, there is provided an adenoviral vector including a DNA sequence encoding a lung surfactant protein.

In one embodiment, the adenoviral vector is a replication deficient adenoviral vector, i.e., such vector is free of a DNA sequence(s) which is (are) required for viral replication, such as, for example, the E1 DNA sequence or a portion thereof. In one embodiment, the adenoviral vector is free of at least a portion of the adenoviral E1 DNA sequence and is free of at least a portion of the adenoviral E3 DNA sequence. The E3 region encodes several polypeptides which help the adenovirus to evade the immune surveillance of the host.

In one embodiment, the adenoviral vector comprises an adenoviral 5' inverted terminal repeat, or ITR; an adenoviral 3'

ITR; an adenoviral encapsidation signal; the DNA sequence encoding a lung surfactant protein; and a promoter controlling the expression of the DNA sequence encoding the lung surfactant protein. The vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and is not free of DNA sequences encoding adenoviral proteins expressed by the adenoviral major late promoter. In one embodiment, the vector is also free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences. In another embodiment, the vector is free of the adenoviral E1 and E3 DNA sequences, and is free of one of the E2 and E4 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

In yet another embodiment, the vector is free of at least the majority of the E1 and E3 DNA sequences, is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences, and is free of DNA sequences encoding adenoviral proteins expressed under control of the adenoviral major late promoter.

The DNA sequence encoding a lung surfactant protein is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus promoter; the respiratory

syncytial virus promoter; inducible promoters, such as the mouse mammary tumor virus, or MMTV, promoter; the metallothionein promoter; and heat shock promoters. In addition, tissue-specific promoters such as, but not limited to, lung surfactant protein promoters, may also be employed. It is to be understood, however, that the scope of the present invention is not to be limited to any specific promoter.

Lung surfactant proteins which may be encoded by the DNA sequence encoding a lung surfactant protein include surfactant protein A (SPA), surfactant protein B (SPB), and surfactant protein C (SPC).

Surfactant protein A is described in Kuroki, et al., J. Biol. Chem., Vol. 263, No. 7, pgs. 3388-3394 (March 5, 1988). Surfactant protein B and DNA encoding therefor are described in Pilot-Matias, et al., DNA, Vol. 8, No. 2, pgs. 75-86 (1989), Glasser, et al., Proc. Nat. Acad. Sci., Vol 84, pgs. 4007-4011 (June 1987); Revak, et al., J. Clin. Invest., Vol. 81, pgs. 826-833 (March 1988); O'Reilly, et al., Biochimica et Biophysica Acta, Vol. 1011, pgs. 140-148 (1989); and Weaver, et al., J. Amer. Phys. Soc., pgs. 982-987 (1988). Surfactant protein C is described further in Glasser, et al., J. Biol. Chem., Vol. 263, No. 21, pgs. 10326-10331 (July 25, 1988).

In one embodiment, the DNA sequence encoding a lung surfactant protein encodes lung surfactant protein B. Present evidence suggests that SPB is the most clinically important lung surfactant protein of those herein above described.

Such a vector, in a preferred embodiment, is assembled first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the leftward adenoviral genomic elements, the "critical left end elements", which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and the Ela enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a tripartite leader sequence, a multiple cloning site; a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. Such DNA segment serves as a substrate for homologous recombination with a modified or mutated adenovirus, and such sequence may encompass, for example, a segment of the adenoviral genome from base 3328 to base 6241 of the adenovirus 5 genome. The plasmid may also include a selectable marker and an origin of replication. The origin of replication, may be, for example, a bacterial origin of replication. A representative example of such a shuttle plasmid is pAVS6, shown in Figure 4. An intron may be included within the transcribed portion to enhance the cytoplasmic mRNA accumulation levels.

The multiple cloning site facilitates the insertion of the DNA sequence encoding a lung surfactant protein into the plasmid. The DNA sequence encoding the lung surfactant protein may be inserted into the multiple cloning site. In general, restriction enzyme sites separating the above-mentioned components of the shuttle plasmid include "rare" restriction enzyme sites; i.e., sites which are found to occur randomly in

eukaryotic genes at a frequency from about one in every 10,000 to about one in every 100,000 base pairs. This increases the flexibility and ease of rearranging components of the vectors in assembled shuttle plasmids.

Homologous recombination is then effected with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted, as shown, for example, in Figure 8. Such homologous recombination may be effected through co-transfection of the shuttle plasmid and the modified adenovirus into a helper cell line, such as 293 (embryonic kidney epithelial) cells, by CaPO₄ precipitation. Upon such homologous recombination, a cloning vector is formed in which the modified adenovirus DNA which was 5' to the DNA segment in the shuttle plasmid corresponding to a similar segment of the modified adenoviral genome is replaced with the components in the shuttle plasmid which are 5' to such DNA segment. This homologous recombination, or "crossing over" event, can occur anywhere along the segment of the genome of the modified adenovirus which corresponds to the segment which is also contained within the shuttle plasmid (such as, for example, bases 3328 to 6241 of adenovirus 5 in Example 1 shown below).

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR; an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; a tripartite leader sequence; a DNA sequence encoding a lung surfactant protein; a poly A signal; adenoviral DNA free of at least the majority of

the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. This vector may then be introduced into a cell line such as the 293 cell line for production of large amounts of infectious recombinant adenoviral particles. The 293 cell line is a human fetal kidney epithelial cell line into which has been permanently introduced 11% of the left end of the adenovirus 5 genome. This directs the synthesis of the adenoviral E1a and E1b proteins and allows trans-complementation of E1-deleted vectors.

The infectious viral particles may then be administered to a host in vivo as part of a gene therapy procedure. Such infectious viral particles may be administered systemically, such as by intravenous or intraperitoneal or intramuscular or subcutaneous administration, or may be administered topically, such as by intratracheal or intrabronchial administration, or, alternatively, the infectious viral particles may be administered in an aerosol formulation. The infectious viral particles may be administered in an amount of up to about 10^{13} pfu, preferably from about 10^7 pfu to about 10^{12} pfu. For example, the infectious viral particles may be employed in the transduction of the epithelium of the respiratory tract or alveoli; whereby the lung epithelial cells will express lung surfactant protein in amounts sufficient to achieve clinical correction of lung surfactant protein deficiency.

In addition, the infectious viral particles may be used to transduce eukaryotic cells in vitro. Eukaryotic cells which may be transduced include, but are not limited to, macrophages,

lymphocytes, fibroblasts, liver cells, bronchial cells, and other epithelial or endothelial cells. Such eukaryotic cells then may be administered to a host as part of a gene therapy procedure, or may be cultured in vitro whereby such cells produce lung surfactant protein.

In addition, the infectious viral particles may be used to transduce eukaryotic cells in vitro for the in vitro production of lung surfactant protein. Examples of eukaryotic cells which may be transduced in vitro for the in vitro production of lung surfactant protein include, but are not limited to, those eukaryotic cells hereinabove described, as well as Chinese Hamster Ovary (CHO) cells, COS-7 cells, NIH 3T3 cells, vero cells, HeLa cells, MRC-5 cells, CN1 cells, W138 cells, and chicken lymphoma cells. The lung surfactant protein produced by such cells may then be administered to a host in conjunction with an acceptable pharmaceutical carrier in order to treat lung surfactant protein deficiency states.

In another embodiment, the vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding a lung surfactant protein; and a promoter controlling the DNA sequence encoding a lung surfactant protein. The vector is free of the adenoviral E1, E2, E3, and E4 DNA sequences, and the vector is free of DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter; i.e., the vector is free of DNA encoding adenoviral

structural proteins. Such vector is sometimes hereinafter referred to as a "gutless adenoviral vector," or "GLAd" vector.

Promoters which are contained in the vector may be those hereinabove described.

Such vectors may be constructed by removing the adenoviral 5' ITR, the adenoviral 3' ITR, and the adenoviral encapsidation signal, from an adenoviral genome by standard techniques. Such components, as well as a promoter (which may be an adenoviral promoter or a non-adenoviral promoter), tripartite leader sequence, poly A signal, may, by standard techniques, be ligated into a base plasmid or "starter" plasmid such as, for example, pKSII(Stratagene), to form an appropriate cloning vector. The cloning vector may include a multiple cloning site, as hereinabove described, to facilitate the insertion of the foreign DNA sequence into the cloning vector. An appropriate vector in accordance with the present invention is thus formed by cutting the cloning vector by standard techniques at appropriate restriction sites in the multiple cloning site, and then ligating the DNA sequence encoding a lung surfactant protein into the cloning vector.

The GLAd vector may then be packaged into infectious viral particles using a helper adenovirus or cell line which provides the necessary packaging materials. If a helper virus is used, in one embodiment, preferably it has a defective encapsidation signal in order that the helper virus will not package itself. Examples of such encapsidation-defective helper viruses which may

be employed are described in Grable, et al., J. Virol., Vol. 66, pgs. 723-731 (1992), and in Grable, et al., J. Virol., Vol. 64, pgs. 2047-2056 (1990). In another embodiment, the helper virus has a normal packaging signal.

DNA for the vector and the encapsidation-defective helper virus are transfected into an appropriate cell line for the generation of infectious viral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes. Examples of appropriate cell lines include, but are not limited to, HeLa cells, A549 cells, or 293 (embryonic kidney epithelial) cells. The infectious viral particles may then be purified away from helper virus by CsCl isopycnic density centrifugation and transduced into lung epithelial cells lining the respiratory tract or alveoli, as hereinabove described, whereby such cells express lung surfactant protein.

In another alternative, the vector is transfected into the cells, followed by infection of the cells with the encapsidation-defective helper virus.

The invention will now be described with respect to the following example, however, the scope of the present invention is not intended to be limited thereby.

Example 1

The adenoviral construction shuttle plasmid pAVS6 was constructed in several steps using standard cloning techniques including polymerase chain reaction based cloning techniques.

First, the 2913 bp BglIII, HindIII fragment was removed from Ad-d1327 and inserted as a blunt fragment into the XhoI site of pKSII (Stratagene, La Jolla, CA) (Figure 1). Ad-d1327 (Thimmappaya, et al., Cell, Vol. 31, pg. 543 (1983)) is identical to adenovirus 5 except that an XbaI fragment including bases 28593 to 30470 (or map units 78.5 to 84.7) of the adenovirus 5 genome, and which is located in the E3 region, has been deleted.

The complete Adenovirus 5 genome is registered as Genbank accession #M73260, incorporated herein by reference, and the virus is available from the American Type Culture Collection, Rockville, Maryland, U.S.A. under accession number VR-5.

Ad-d1327 was constructed by routine methods from Adenovirus 5 (Ad5). The method is outlined briefly as follows and previously described by Jones and Shenk, Cell 13:181-188 (1978). Ad5 DNA is isolated by proteolytic digestion of the virion and partially cleaved with Xba I restriction endonuclease. The Xba I fragments are then reassembled by ligation as a mixture of fragments. This results in some ligated genomes with a sequence similar to Ad5, except excluding sequences 28593 bp to 30470 bp. This DNA is then transfected into suitable cells (e.g. KB cells, HeLa cells, 293 cells) and overlaid with soft agar to allow plaque formation. Individual plaques are then isolated, amplified, and screened for the absence of the 1878 bp E3 region Xba I fragment. The orientation of this fragment was such that the BglIII site was nearest the T7 RNA polymerase site of pKSII.

and the HindIII site was nearest the T3 RNA polymerase site of pKSII. This plasmid was designated pHR. (Figure 1).

Second, the ITR, encapsidation signal, Rous Sarcoma Virus promoter, the adenoviral tripartite leader (TPL) sequence and linking sequences were assembled as a block using PCR amplification (Figure 2). The ITR and encapsidation signal (sequences 1-392 of Ad-d1327 [identical to sequences from Ad5, Genbank accession #M73260]) were amplified (amplification 1) together from Ad-d1327 using primers containing NotI or AscI restriction sites. The Rous Sarcoma Virus LTR promoter was amplified (amplification 2) from the plasmid pRC/RSV (sequences 209 to 605; Invitrogen, San Diego, CA) using primers containing an AscI site and an SfiI site. DNA products from amplifications 1 and 2 were joined using the "overlap" PCR method (amplification 3) with only the NotI primer and the SfiI primer. Complementarity between the AscI containing end of each initial DNA amplification product from reactions 1 and 2 allowed joining of these two pieces during amplification. Next the TPL was amplified (amplification 4) (sequences 6049 to 9730 of Ad-d1327 [identical to similar sequences from Ad5, Genbank accession #M73260]) from cDNA made from mRNA isolated from 293 cells infected for 16 hours with Ad-d1327 using primers containing SfiI and XbaI sites respectively. DNA fragments from amplification reactions 3 and 4 were then joined using PCR (amplification 5) with the NotI- and XbaI-site-containing primers, thus creating the complete gene block.

Third, the ITR-encapsidation signal-TPL fragment was then purified, cleaved with NotI and XbaI and inserted into the NotI, XbaI cleaved pHR plasmid. This plasmid was designated pAVS6A and the orientation was such that the NotI site of the fragment was next to the T7 RNA polymerase site (Figure 3).

Fourth, the SV40 early polyA signal was removed from SV40 DNA as an HpaI-BamHI fragment, treated with T4 DNA polymerase and inserted into the SalI site of the plasmid pAVS6A-(Figure 3) to create pAVS6 (Figures 3 and 4).

A 2kb DNA fragment containing the whole human pulmonary surfactant protein B (SPB) cDNA (Figure 5) (Pilot-Matias, 1989) was obtained from plasmid pKC4-SPB (Figure 6) (Weaver, et al., J. Amer. Phys. Soc., pgs. L-95 to L-103 (1992)) by EcoRI digestion. This DNA fragment was isolated, purified, and then cloned into the EcoRV site of plasmid pAVS6. (Figure 4). Three identical clones with correct insertion of SPB cDNA were obtained. Such clones are named pAVS6-SPB#7, pAVS6-SPB#12, and pAVS6-SPB#13. pAVS6-SPB#7 is shown in Figure 7. The orientation of the SPB DNA within the shuttle plasmid was obtained by evaluating the DNA sequences of the two termini of the SPB cDNA insert in the plasmid with primers derived from pAVS6.

The recombinant adenoviral vector AV1SPB1 (Figure 8), containing SPB cDNA was constructed through homologous recombination between the Ad5 deletion mutant Ad-d1327 (Figure 8), and pAVS6-SPB#7. Homologous recombination, or "crossing over," occurs between Ad-d1327 and pAVS6-SPB#7, along the

segment common to both Ad-d1327 and pAVS6.SPB#7 which corresponds to bases 3328 to 6241 (or map units 9.24 to 17.34) of the adenovirus 5 genome. Ad-d1327 has a deleted E3 region in which base pairs 28593 to 30470 are absent (Thimmappaya, et al, Cell, Vol. 31, pgs. 543-551 (1982)). pAVS6.SPB#7 contains an adenoviral 5' ITR, an origin of replication contained completely within the 5' ITR, an Ela enhancer and encapsidation signal, a Rous Sarcoma Virus promoter, an adenovirus 5 tripartite leader sequence and the 2kb human SPB cDNA including the entire protein coding sequence (nucleotides 1 to 1172), and the SV40 poly A signal.

Example 2

293 cells (ATCC No. CRL 1573) were infected with AV1SPB1 at a multiplicity of infection (MOI) of 50 MOI units. At 12 hours post-infection, the cells were radiolabeled with ³⁵S-methionine (50μCi/ml) overnight. Identical amounts of labeled protein were used for immunoprecipitation with antisera against SPB. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis on 16% gel. The gels were fluorographed. C¹⁴-labeled molecular weight markers and BioRad broad range molecular weight markers were used as size markers. As shown in Figure 9, lane 1 shows an uninfected control; lane 2 shows AV1SPB1-infected 293 cells in which electrophoresis of immunoprecipitates occurred under reducing conditions; and lane 3 shows AV1SPB1-infected 293 cells in which electrophoresis of immunoprecipitates occurred under non-reducing conditions. The active SPB peptide migrates at approximately Mr=6,000 to 8,000 (reduced) and forms oligomers

(unreduced) identical to that of native human SPB ($M_r=18,000$). The precursor protein also was detected in both reduced and unreduced conditions.

Example 3

Mouse lung Type II-like epithelial cell lines were transduced with adenoviral vector AV1SPB1, which contains the full length human SPBcDNA under the control of the Rous Sarcoma Virus promoter. Expression of SPB was assessed by RNase protection using ^{32}P -labelled probes specific for endogenous mouse SPB (upper band) or human SPB (middle band). A B-tubulin specific probe (lowest band) also was used to ensure that the same amount of RNA was added to each assay. The B-tubulin probe did not recognize human B-tubulin in lane 1. As shown in Figure 10, cells infected with AV1SPB1 at 50 multiplicity of infection (MOI) units (lane 2), 100 MOI units (lane 3), and 150 MOI units (lane 4), clearly expressed human SPB mRNA. Human SPB was not detected in uninfected cells (lane 5).

Example 4

Four cotton rats were anesthetized by metaphane, and were given 1.5×10^{10} pfu of AV1SPB1. The rats were sacrificed at 2 (n=1), 3 (n=2) or 45 (n=1) days after administration, and the lungs were harvested. Total lung RNA then was extracted using the guanidine thiocyanate-CsCl technique (Chirgwin, et al., Biochemistry, Vol. 18, pgs. 5294-5299 (1979)). Total lung RNA (15 μg) was subjected to formaldehyde agarose gel electrophoresis and transferred to a nylon membrane (Nytran,

Schleicher and Schuell). The filter was crosslinked (UV Crosslink, Stratagene), and hybridized with a ^{32}P -labeled 2.0 kb human SP-B cDNA probe prepared by random priming (Loftstrand) and evaluated by autoradiography. Lungs from uninfected control rats and from rats infected with Av1LacZ4 also were subjected to the above hybridization procedure.

Northern blot analysis, as shown in Figure 11, demonstrated that human SP-B mRNA was expressed in the lungs of cotton rats infected with Av1SP-B1, but not in those of uninfected animals nor in animals infected with Av1LacZ4. A 2.0 kb mRNA for the human SP-B gene was detected in cotton rats infected with Av1SP-B1, consistent with the size of the full length human SP-B mRNA transcribed by the vector.

Example 5

Two cotton rats were anesthetized with metaphane, and Av1SP-B1 (diluted in PBS to 10^{10} pfu/300 μl) was administered via intranasal instillation. Two days after infection, the rats were sacrificed and the lungs were harvested. The lungs were washed twice in PBS and perfused with methionine-free LHC-8 medium (Biofluids), minced, and incubated for 24 hours in medium with ^{35}S -Cys/Met (1ml of medium, 100 $\mu\text{Ci/ml}$). The presence of SP-B in media and lysed lung explants was assessed by immunoprecipitation with rabbit anti-human SP-B antiserum to detect the secreted SP-B and processed SP-B peptides. (Weaver, et al., Am. J. Physiol., Vol. 263, pgs. L95-L103 (1992)). Av1LacZ4 treated rats and untreated rats were used as controls.

As shown in Figure 12, de novo synthesis and secretion of human SP-B peptide was detected from lung fragments removed from animals infected with Av1SPB1, and was not detected in uninfected animals or in animals infected with Av1LacZ4. Human SP-B peptide was detected as secreted, with 8 kda and 18 kda oligomeric forms suggesting that vector derived precursor SP-B (proSP-B) was processed after in vivo adenoviral vector-mediated gene transfer.

Example 6

Cotton rats were treated intranasally with Av1SP-B1 in amounts of (a) 0.5×10^9 pfu (n=1); (b) 1.5×10^9 pfu (n=1); or (c) 1.5×10^{10} pfu (n=1). The animals were sacrificed 48 hours after infection, and the lungs were prepared for in situ hybridization analysis according to the method of Wert, et al., Development Biology, Vol. 156, pgs. 426-443 (1993), using human SP-BcRNA. An uninfected rat was used as a control.

Lung sections were inflation fixed in 20 cm of water in 4% paraformaldehyde at 4°C overnight. Radiolabeled ribroprobe for human SP-B was generated by in vitro transcription in the presence of ^{35}S -VTP. Slides were hybridized to the human SP-B probe under stringent conditions and exposed to emulsion from one to eight days, after which the slides were counterstained with hematoxylin and eosin. Antisense and sense probes were compared in Av1SPB1-treated, in Av1LacZ4-treated, and in control (untreated) animals.

In situ hybridization of the Av1SP-B1 infected cotton rats demonstrated expression of human SP-B mRNA in bronchiolar and

alveolar epithelial cells. Human SP-B mRNA was detected in a patchy distribution and increased in a dose-dependent manner, as shown in Figures 13 through 16, which show the in situ hybridization results of the rats treated with 0.5×10^9 pfu of Av1SP-B1; 1.5×10^9 pfu of Av1SP-B1; 1.5×10^{10} pfu of Av1SP-B1; and an uninfected control rat, respectively. Light microscopic analysis of the lungs of Av1SP-B1 or Av1LacZ treated animals demonstrated a mild inflammatory response with a peribronchiolar lymphomonocytic infiltrate, increased macrophages, and some polymorpholeukocytes. The infiltrates were prominent after 48 hours and were dose-dependent. In parallel experiments with Av1lac24, the infiltrates were essentially resolved 3 to 4 weeks after exposure.

All patents, publications, and database entries referenced in this specification are indicative of the level of skill of persons in the art to which the invention pertains. The disclosures of all such patents, publications (including published patent applications), and database entries are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced

other than as particularly described and still be within the scope of the accompanying claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Trapnell, Bruce
Whitsett, Jeffrey
- (ii) TITLE OF INVENTION: Adenoviral Vectors Including
DNA Encoding Lung Surfactant
Protein
- (iii) NUMBER OF SEQUENCES: 1
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Carella, Byrne, Bain, Gilfillan,
Cecchi, Stewart, & Olstein
 - (B) STREET: 6 Becker Farm Road
 - (C) CITY: Roseland
 - (D) STATE: New Jersey
 - (E) COUNTRY: USA
 - (F) ZIP: 07068
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 3.5 inch diskette
 - (B) COMPUTER: IBM PS/2
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: Word Perfect5.1

- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:

- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/044,406
 - (B) FILING DATE: 08-APR-1993

- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Olstein, Elliot M.
 - (B) REGISTRATION NUMBER: 24,025
 - (C) REFERENCE/DOCKET NUMBER: 271010-164

- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 201-994-1700
 - (B) TELEFAX: 201-994-1744

- (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2,016 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

 - (ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

(A) NAME/KEY: Surfactant protein B cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTCCGTCA	AGCTGCAGAG	GTGCCATGGC	TGAGTCACAC	CTGCTGCAGT	GGCTGCTGCT	60
TTAAGGCAGT	TCGACGTCTC	CACGGTACCG	ACTCAGTGTG	GACGACGTCA	CCGACGACGA	
GCTGCTGCCC	ACGCTCTGTG	GCCCAGGCAC	TGCTGCCTGG	ACCACCTCAT	CCTTGGCCTG	120
CGACGACGGG	TGCGAGACAC	CGGTCCGTG	ACGACGGACC	TGGTGGAGTA	GGAACCGGAC	
TGCCCAGGGC	CCTGAGTTCT	GGTGCCAAAG	CCTGGAGCAA	GCATTGCAGT	GCAGAGCCCT	180
ACGGGTCCCG	GGACTCAAGA	CCACGGTTTC	GGACCTCGTT	CGTAACGTCA	CGTCTCGGGA	
AGGGCATTGC	CTACAGGAAG	TCTGGGGACA	TGTGGGAGCC	GATGACCTAT	GCCAAGAGTG	240
TCCCGTAACG	GATGTCCTTC	AGACCCCTGT	ACACCCCTCG	CTACTGGATA	CGGTTCTCAC	
TGAGGACATC	GTCCACATCC	TTAACAAGAT	GGCCAAGGAG	GCCATTTTCC	AGGACACGAT	300
ACTCCTGTAG	CAGGTGTAGG	AATTGTTCTA	CCGGTTCCTC	CGGTAAAAGG	TCCTGTGCTA	
GAGGAAGTTC	CTGGAGCAGG	AGTGCAACGT	CCTCCCCTTG	AAGCTGCTCA	TGCCCCAGTG	360
CTCCTTCAAG	GACCTCGTCC	TCACGTTGCA	GGAGGGGAAC	TTCGACGAGT	ACGGGGTCAC	
CAACCAAGTG	CTTGACGACT	ACTTCCCCCT	GGTCATGGAC	TACTTCCAGA	ACCAGACTGA	420
GTTGGTTCAC	GAACTGCTGA	TGAAGGGGGA	CCAGTAGCTG	ATGAAGGTCT	TGGTCTGACT	
CTCAAACGGC	ATCTGTATGC	ACCTGGGCCT	GTGCAAATCC	CGGCAGCCAG	AGCCAGAGCA	480
GAGTTTGCCG	TAGACATACG	TGGACCCGGA	CACGTTTAGG	GCCGTCCGTC	TCGGTCTCGT	
GGAGCCAGGG	ATGTCAGACC	CCCTGCCCAA	ACCTCTGCGG	GACCCCTCTG	CAGACCCTCT	540
CCTCGGTCCC	TACAGTCTGG	GGGACGGGTT	TGGAGACGCC	CTGGGAGACG	GTCTGGGAGA	
GCTGGACAAG	CTCGTCTCTC	CTGTGCTGCC	CGGGGCCCTC	CAGGCGAGGC	CTGGGCCTCA	600
CGACCTGTTC	GAGCAGGAGG	GACACGACGG	GCCCCGGGAG	GTCCGCTCCG	GACCCGGAGT	
CACACAGGAT	CTCTCCGAGC	AGCAATTCCC	CATTCTCTC	CCCTATTGCT	GGCTCTGCAG	660
GTGTGTCCTA	GAGAGGCTCG	TCGTTAAGGG	GTAAGGAGAG	GGGATAACGA	CCGAGACGTC	
GGCTCTGATC	AAGCGGATCC	AAGCCATGAT	TCCCAAGGGT	GCGCTACGTG	TGGCAGTGGC	720
CCGAGACTAG	TTCGCCTAGG	TTCGGTACTA	AGGGTTCCCA	CGCGATGCAC	ACCGTCACCG	
CCAGGTGTGC	CGCGTGGTAC	CTCTGGTGGC	GGGCGGCATC	TGCCAGTGCC	TGGCTGAGCG	780
GGTCCACACG	GCGCACCATG	GAGACCACCG	CCC GCCGTAG	ACGGTCACGG	ACCGACTCGC	
CTACTCCGTC	ATCCTGCTCG	ACACGCTGCT	GGGCCGCATG	CTGCCCCAGC	TGGTCTGCCG	840
GATGAGGCAG	TAGGACGAGC	TGTGCGACGA	CCCGGCGTAC	GACGGGGTCG	ACCAGACGGC	
CCTCGTCTCT	CGGTGCTCCA	TGGATGACAG	CGCTGGCCCA	AGGTCGCCGA	CAGGAGAATG	900
GGAGCAGGAG	GCCACGAGGT	ACCTACTGTC	GCGACCGGGT	TCCAGCGGCT	GTCTCTTAC	
GCTGCCGCGA	GACTCTGAGT	GCCACCTCTG	CATGTCCGTG	ACCACCCAGG	CCGGGAACAG	960
CGACGGCGCT	CTGAGACTCA	CGGTGGAGAC	GTACAGGCAC	TGGTGGGTCC	GGCCCTTGTC	
CAGCGAGCAG	GCCATACTAC	AGGCAATGCT	CCAGGCCTGT	GTTGGCTCCT	GGCTGGACAG	1020
GTCGCTCGTC	CGGTATGATG	TCCGTTACGA	GGTCCGGACA	CAACCGAGGA	CCGACCTGTC	

GGAAAAGTGC	AAGCAATTG	TGGAGCAGCA	CACGCCCCAG	CTGCTGACCC	TGGTGCCAG	1080
CCTTTTCACG	TTCGTTAAAC	ACCTCGTCGT	GTGCGGGGTC	GACGACTGGG	ACCACGGGTC	
GGGCTGGGAT	GCCCCACCCA	CCTGCCAGGC	CCTCGGGGTG	TGTGGGACCA	TGTCCAGCCC	1140
CCCGACCCTA	CGGGTGTGGT	GGACGGTCCG	GGAGCCCCAC	ACACCCTGGT	ACAGGTGGG	
TCTCCAGTGT	ATCCACAGCC	CCGACCTTTG	ATGAGAACTC	AGCTGTCCAG	GTGCAAGGA	1200
AGAGGTCACA	TAGGTGTCCG	GGCTGGAAC	TACTCTTGAG	TCGACAGGTC	CACGTTTCCT	
AAAGCCAAAGT	GAGAGGGGCT	CTGGGACCAT	GGTGACCAGG	CTCTTCCCCT	GCTCCCTGGC	1260
TTTCGGTTCA	CTCTCCCCGA	GACCCTGGTA	CCACTGGTCC	GAGAAGGGGA	CGAGGGACCG	
CCTCGCCAGC	TGCCAGGCTG	AAAAGAAGCC	TCAGTCCCA	CACCGCCCTC	CTCACCGCCC	1320
GGAGCGGTG	ACGGTCCGAC	TTTTCTTCGG	AGTCGAGGGT	GTGGCGGGAG	GAGTGGCGGG	
TTCTCTGGCA	GTCACTTCCA	CTGGTGGACC	ACGGGCCCCC	AGCCCTGTGT	CGGCCTTGTC	1380
AAGGAGCCGT	CAGTGAAGGT	GACCACCTGG	TGCCCCGGGG	TCGGGACACA	GCCGGAACAG	
TGTCTCAGCT	CAACCACAGT	CTGACACCAG	AGCCCACTTC	CATCCTCTCT	GGTGTGAGGC	1440
ACAGAGTCGA	GTTGGTGTCA	GACTGTGTGTC	TCGGGTGAAG	GTAGGAGAGA	CCCACTCCG	
ACAGCGAGGG	CAGCATCTGG	AGGAGCTCTG	CAGCCTCCAC	ACCTACCACG	ACCTCCCAGG	1500
TGTCGCTCCC	GTCGTAGACC	TCCTCGAGAC	GTCGGAGGTG	TGGATGGTGC	TGGAGGGTCC	
GCTGGGCTCA	GGAAAAACCA	GCCACTGCTT	TACAGGACAG	GGGGTTGAAG	CTGAGCCCCG	1560
CGACCCGAGT	CCTTTTGGT	CGGTGACGAA	ATGTCCTGTC	CCCCAACTTC	GA CTGGGGC	
CCTCACACCC	ACCCCATGTC	ACTCAAAGAT	TGGATTTTAC	AGCTACTTGC	AATTCAAAT	1620
GGAGTGTGGG	TGGGGGTACG	TGAGTTTCTA	ACCTAAATG	TCGATGAACG	TTAAGTTTTA	
TCAGAAGAAT	AAAAATGGG	AACATACAGA	ACTCTAAAG	ATAGACATCA	GAAATTGTTA	1680
AGTCTTCTTA	TTTTTTACCC	TTGTATGTCT	TGAGATTTTC	TATCTGTAGT	CTTTAACAT	
AGTTAAGCTT	TTTCAAAAA	TCAGCAATTC	CCAGCGTAGT	CAAGGGTGGA	CATGCACGCG	1740
TCAATTCGAA	AAAGTTTTTT	AGTCGTAAAG	GGTCGCATCA	GTTCCACCT	GTACGTGCGC	
TCTGGCATGA	TGGGATGGCG	ACCGGGCAAG	CTTTCTTCCT	CGAGATCGTC	TGCTGCTTGA	1800
AGACCGTACT	ACCCTACCGC	TGGCCCGTTC	GAAAGAAGGA	GCTCTAGCAG	ACGACGAACT	
GAGCTATTGC	TTTGTTAAGA	TATAAAAAGG	GGTTTCTTTT	TGTCTTTCTG	TAAGGTGGAC	1860
CTCGATAACG	AAACAATTCT	ATATTTTCC	CCAAAGAAAA	ACAGAAAGAC	ATTCCACCTG	
TTCCAGCTTT	TGATTGAAAG	TCCTAGGGTG	ATTCTATTTT	TGCTGTGATT	TATCTGCTGA	1920
AAGGTCGAAA	ACTAACTTTC	AGGATCCCAC	TAAGATAAAG	ACGACACTAA	ATAGACGACT	
AAGCTCAGCT	GGGGTTGTGC	AAGCTAGGGA	CCCATTCTTA	TGTAATACAA	TGTCTGCACC	1980
TTCGAGTCGA	CCCCAACACG	TTCGATCCCT	GGGTAAGGAT	ACATTATGTT	ACAGACGTGG	
AATGCTAATA	AAGTCCTATT	CTCTTTTATC	GGAATT			2016
TTACGATTAT	TTCAGGATAA	GAGAAAATAG	CCTTAA			

WHAT IS CLAIMED IS:

1. An adenoviral vector including a DNA sequence encoding a lung surfactant protein.
2. The vector of Claim 1 wherein said lung surfactant protein is Surfactant Protein B.
3. The vector of Claim 1 wherein said adenoviral vector is a replication deficient adenoviral vector.
4. The vector of Claim 3 wherein said adenoviral vector is free of at least a portion of the adenoviral E1 DNA sequence, and is free of at least a portion of the adenoviral E3 DNA sequence.
5. The vector of Claim 4 wherein said vector comprises:
 - an adenoviral 5' ITR;
 - an adenoviral 3' ITR;
 - an adenoviral encapsidation signal;
 - a DNA sequence encoding a lung surfactant protein;
 - a promoter controlling said foreign DNA sequence, said vector being free of at least the majority of the adenoviral E1 and E3 DNA sequences, but not free of all of the E2 and E4 DNA sequences and DNA sequences encoding adenoviral proteins controlled by the adenoviral major late promoter.
6. The vector of Claim 5 wherein the vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences.

7. The vector of Claim 6 wherein said vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of one of the E2 and E4 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

8. The vector of Claim 7 wherein said vector is free of at least the majority of the E1 and E3 DNA sequences, is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences, and is free of DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter.

9. The vector of Claim 5 wherein said promoter is an adenoviral promoter.

10. The vector of Claim 5 wherein said promoter is a heterologous promoter.

11. Infectious viral particles generated from the vector of Claim 1.

12. Lung epithelial cells transfected with the infectious viral particles of Claim 11.

13. Infectious viral particles generated from the vector of Claim 5.

14. Lung epithelial cells transfected with the infectious viral particles of Claim 13.

15. A process for treating lung surfactant protein deficiency states, comprising:

administering to a host an effective amount of the infectious viral particles of Claim 11.

16. A process for treating lung surfactant protein deficiency states, comprising:

administering to a host an effective amount of the infectious viral particles of Claim 13.

17. The process of Claim 15 wherein said infectious viral particles are administered in an amount up to about 10^{13} pfu.

18. The process of Claim 17 wherein said infectious viral particles are administered in an amount of from about 10^7 pfu to about 10^{12} pfu.

19. The process of Claim 16 wherein said infectious viral particles are administered in an amount of from 1 pfu to about 10^{12} pfu.

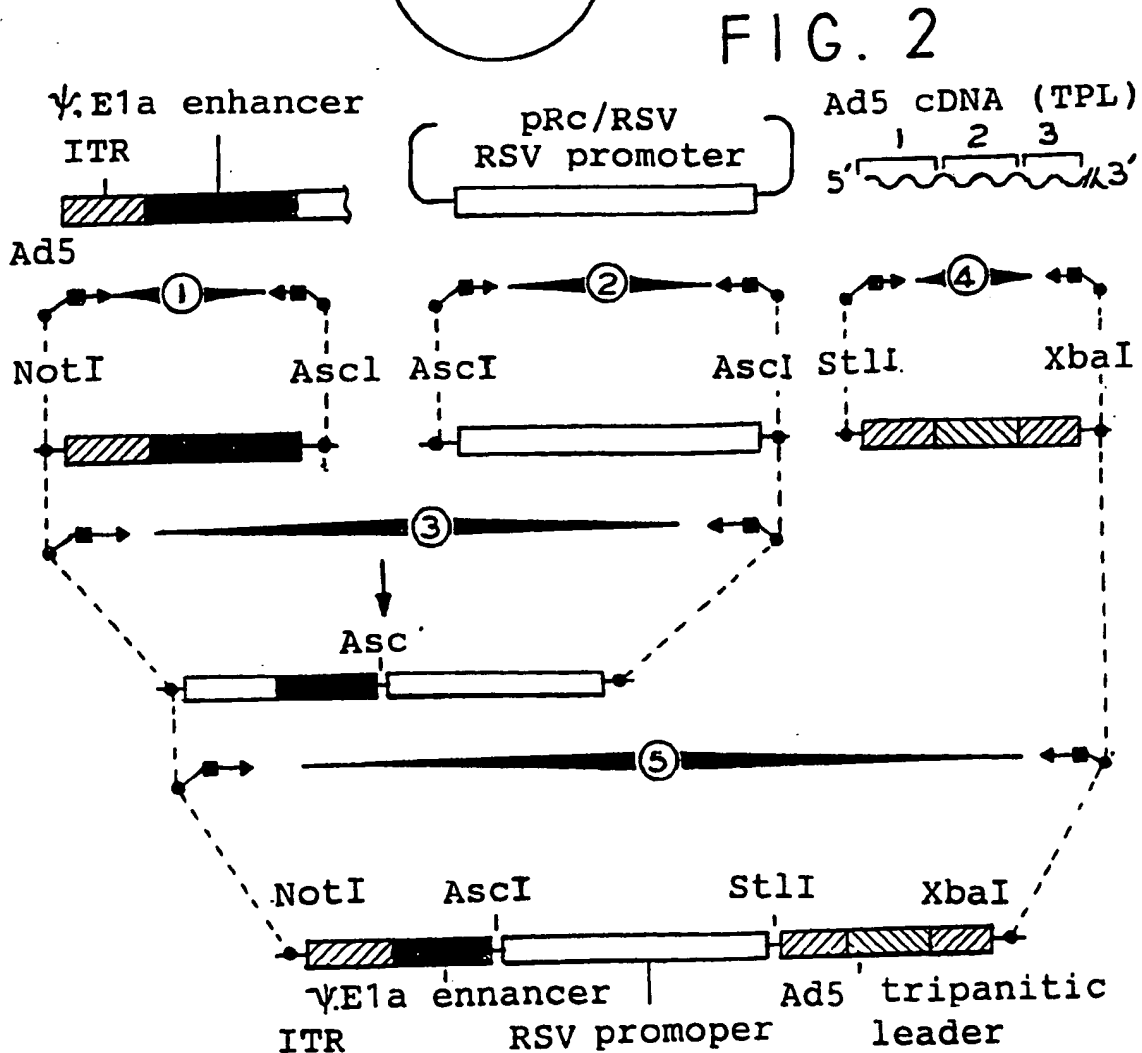
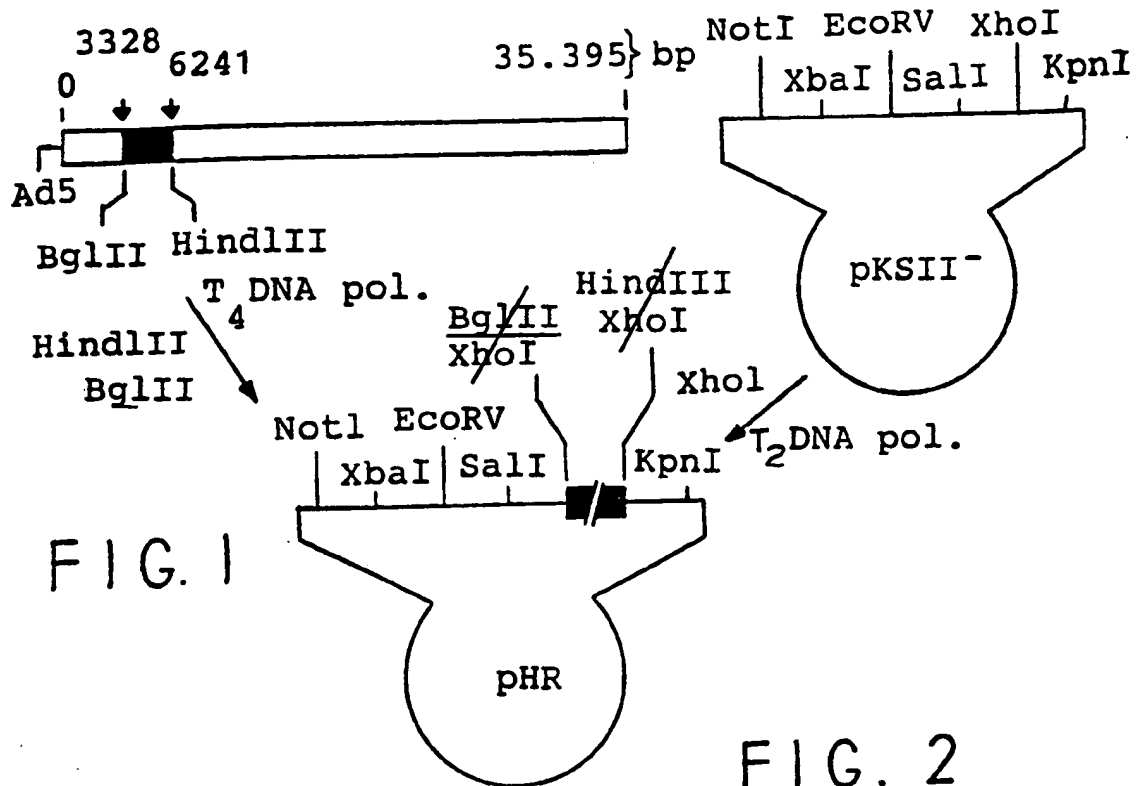
20. The process of Claim 19 wherein said infectious viral particles are administered in an amount of from about 10^7 pfu to about 10^{12} pfu.

21. The process of Claim 15 wherein said lung surfactant protein deficiency state is infant respiratory distress syndrome.

22. The process of Claim 16 wherein said lung surfactant protein deficiency state is infant respiratory distress syndrome.

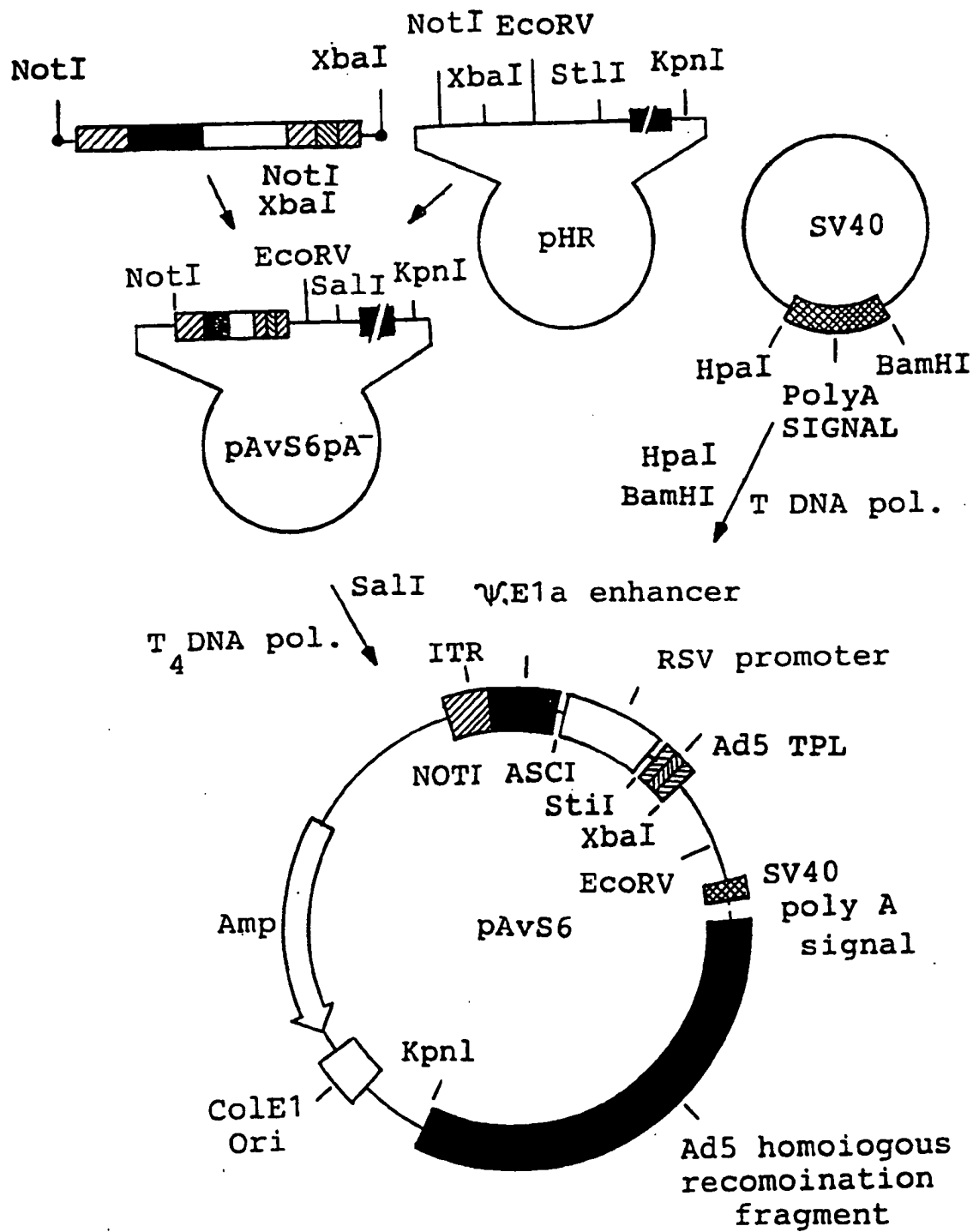
23. The process of Claim 15 wherein said lung surfactant protein deficiency state is adult respiratory distress syndrome.

24. The process of Claim 16 wherein said lung surfactant protein deficiency state is adult respiratory distress syndrome.



2/12

FIG. 3



3/12

FIG. 4

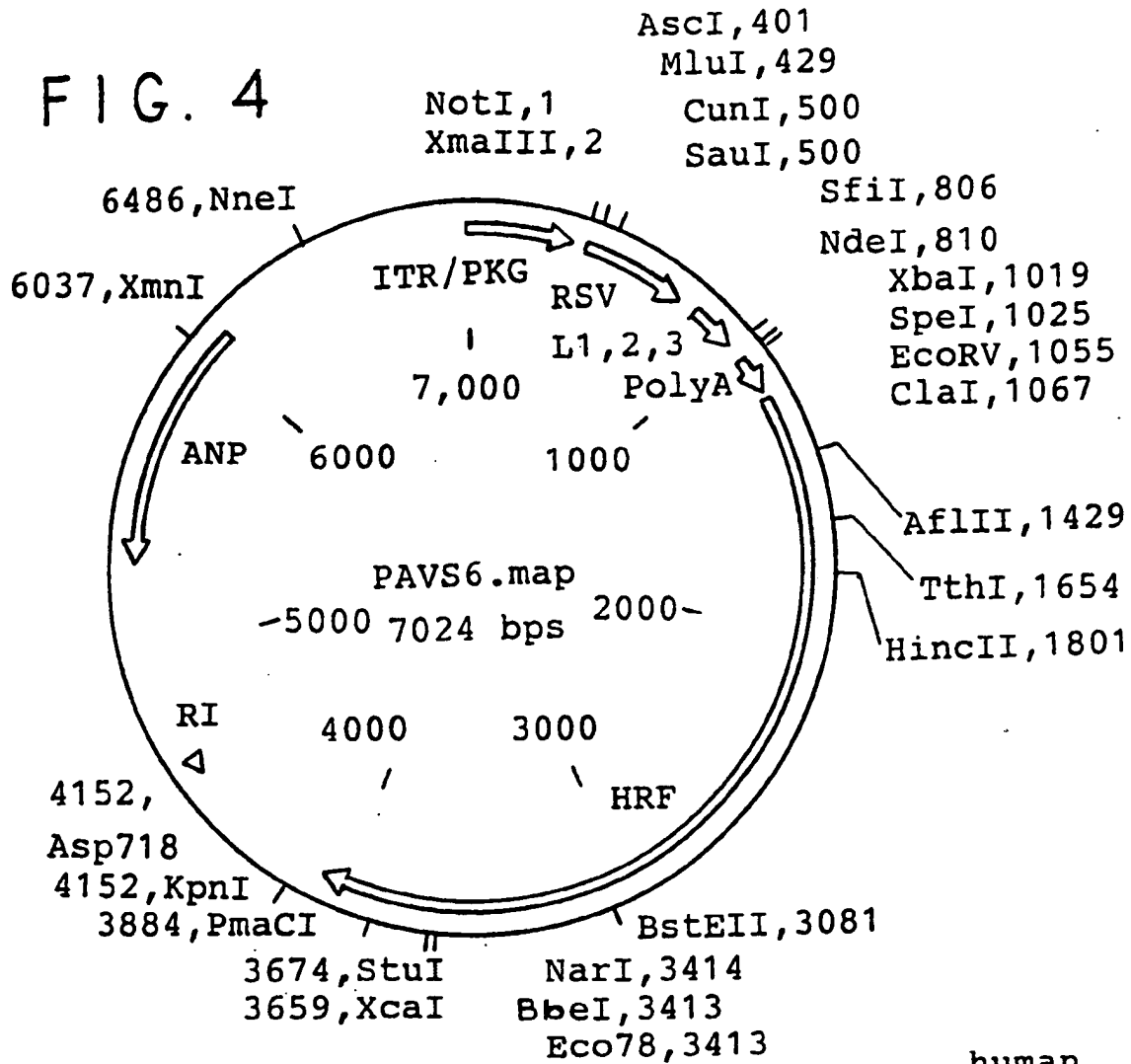


FIG. 6

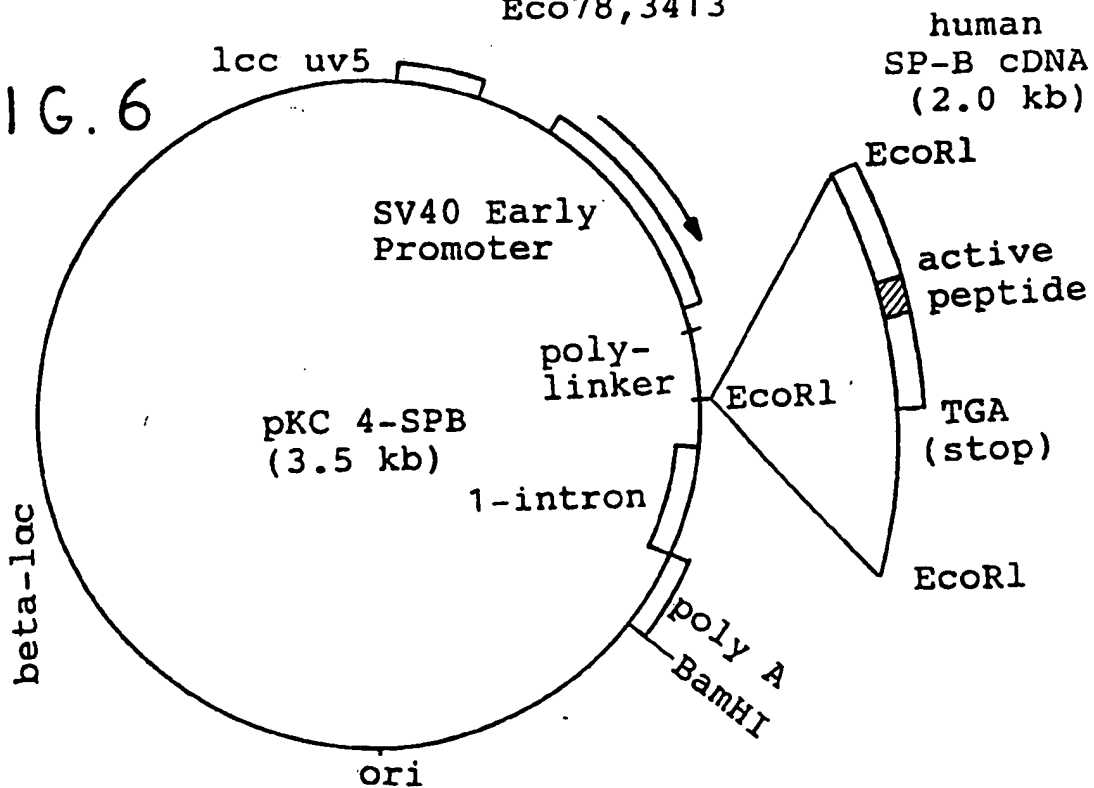


FIG. 5A

* * * S E Q U E N C E * * *

```

1  AATCCGTCA AGCTGCAGAG GTGCCATGGC TGAGTCACAC CTGCTGCAGT GGCTGCTGCT
   TTAAGGCAGT TCGACGTCTC CACGGTACCG ACTCAGTGTG GACGACGTCA CCGACGACGA

61  GGTGCTGCCC ACGCTCTGTG GCCCAGGCAC TGTGCTCTGG ACCACCTCAT CCTTGGCCTG
   CGACGACGGG TGCAGACAC CCGGTCCGTG ACGACGGACC TGGTGGAGTA GGAACCGGAC

121  TGCCCAGGGC CCTGAGTTCT GGTGCCAAG CCTGGAGCAA GCATTGCAGT GCAGAGCCCT
   ACGGTCCCG GACTCAAGA CCACGGTTTC GGACCTCGTT CGTAACGTCA CGTCTCGGA

181  AGGGCATTTC CTACAGGAAG TCTGGGGACA TGTGGGAGCC GATGACCTAT GCCAAGAGTG
   TCCCGTAACG GATGTCCTC AGACCCCTGT ACACCTCGG CTA CTGGATA CGTTCTCAC

241  TGAGGACATC GTCCACATCC TTAACAAGAT GGCCAAGGAG GCCATTTC AGGACACGAT
   ACTCCTGTAG CAGGTGTAGG AATTGTTCTA CCGGTTCCCTC CGGTAAAAGG TCCGTGTGCTA

301  GAGGAAGTTC CTGGAGCAGG AGTGCAACGT CCTCCCCCTG AGCTGCTCA TGCCCCAGTG
   CTCCTTCAAG GACCTCGTCC TCATGTTGCA GGAGGGGAAC TTCGACGAGT ACGGGGTCAC

361  CAACCAAGTG CTTGACGACT ACTTCCCCCT GTCATCGAC TACTTCCAGA ACCAGACTGA
   GTTGGTTCAC GAACTGCTGA TGAAGGGGA CCAGTAGCTG ATGAAGGTCT TGGTCTGACT

421  CTCAAACGGC ATCTGTATGC ACCTGGGCCT GTGCAATCC CGGCAGCCAG AGCCAGAGCA
   GAGTTTGCCG TAGACATACG TGGACCCGGA CACGTTTAGG GCCGTGCGTC TCGGTCTCGT

481  GGAGCCAGGG ATGTCAGACC CCCTGCCCAA ACCTCTGCGG GACCTCTGC CAGACCCCTCT
   CCTCGGTCCC TACAGTCTGG GGGACGGGTT TGGAGACGCC CTGGGAGACG GTCTGGGAGA

```

MATCH WITH FIG. 5B

5/12

FIG. 5B

MATCH WITH FIG. 5A

541	GCTGGACAAG	CTCGTCCCTCC	CTGTGCTGCC	CGGGGCCCTC	CAGGCGAGGC	CTGGGCCTCA
	CGACCTGTTT	GAGCAGGAGG	GACACGACGG	GCCCCGGGAG	GTCCGCTCCG	GACCCGGAGT
601	CACACAGGAT	CTCTCCGAGC	AGCAATTCCC	CATTCCCTCTC	CCCTATTGCT	GGCTCTGCAG
	GTGTGTCCTA	GAGAGGCTCG	TCGTTAAGGG	GTAAGGAGAG	GGGATAACGA	CCGAGACGTC
661	GGCTCTGATC	AAGCGGATCC	AAGCCATGAT	TCCCAAGGGT	GCGCTACGTG	TGGCAGTGCG
	CCGAGACTAG	TTCGCCCTAGG	TTCGGTACTA	AGGGTTCCCA	CGCGATGCAC	ACCGTCACCG
721	CCAGGTGTGC	CGCGTGGTAC	CTCTGGTGCC	GGCGGGCATC	TGCCAGTGCC	TGGCTGAGCG
	GGTCCACACG	GCGCACCATG	GAGACCACCTG	CCCGCCGTAG	ACGGTCACGG	ACCGACTCGC
781	CTACTCCGTC	ATCCTGCTCG	ACACGCTGCT	GGGCCGCGATG	CTGCCCCCAGC	TGGTCTGCCG
	GATGAGGCAG	TAGGACGAGC	TGTGCCGACGA	CCCGCGGTAC	GACGGGGTCG	ACCAGACGGC
841	CCTCGTCCTC	CGGTGCTCCA	TGGATGACAG	CGCTGGCCCCA	AGGTCGCCCGA	CAGGAGAATG
	GGAGCAGGAG	GCCACGAGGT	ACCTACTGTC	GCGACCGGGT	TCCAGCGGCT	GTCCCTCTTAC
901	GCTGCCGCGA	GACTCTGAGT	GCCACCCTCTG	CATGTCCGTG	ACCACCCAGG	CCGGGAACAG
	CGACGGCGCT	CTGAGACTCA	CGGTGGAGAC	GTACAGGCAC	TGGTGGGTCC	GGCCCTTGTC
961	CAGCGAGCAG	GCCATACTAC	AGGCAATGCT	CCAGGCCCTGT	GTTGGCTCCT	GGCTGGACAG
	GTCGCTCGTC	CGGTATGATG	TCCGTTACGA	GGTCCGGACA	ACCCCGAGGA	CCGACCTGTC
1021	GGAAAAGTGC	AAGCAATTG	TGGAGCAGCA	CACGCCCCAG	CTGCTGACCC	TGGTGCCCCAG
	CCTTTTCACG	TTCGTTAAAC	ACCTCGTCGT	GTGCGGGGTC	GACGACTGGG	ACCACGGGTC

MATCH WITH FIG. 5C

6/12

FIG. 5C

MATCH WITH FIG. 5B

1081	GGGCTGGGAT GCCACACCA CCTGCCAGGC CCTUGGGGT TGTGGGACCA TGTCCAGCCC CCCGACCCCTA CGGT TGGT GGACGGTCCG GGAGCCCCCA ACACCTGTT ACAGTCCGG
1141	TCTCCAGTGT ATCCACAGCC CCGACCTTTG ATGAGAACTC AGCTGTCCAG GTGCAAGGA AGAGTCACA TAGGTGTCGG GGCTGGAAAC TACTCTTGAG TCGACAGGTC CACGTTTCCT
1201	AAAGCCAAGT GAGACGGGCT CTGGGACCAT GGTGACCAGG CTCTPCCCTT GCTCCCTGGC TTTCGGTTCA CTCTCCCCGA GACCCTGGTA CCACTGGTCC GAGAAGGGA CGAGGGACCG
1261	CCTCGCCAGC TGCCAGGCTG AAAAGAGCC TCAGCTCCCA GACCGCCCTC CTCACCGCCC GGAGCGGTCG ACGGTCCGAC TTTTCTTCGG AGTCGAGGGT GTGGCGGGAG GAGTGGCGGG
1321	TTCCTCGGCA GTCACCTCCA CTGGTGGACC ACGGGCCCCC AGCCCTGTGT CGGCCTTGTC AAGGAGCCGT CAGTGAAGT GACCACCTGG TGCCCCGGGG TCGGACACA GCCGGAACAG
1381	TGTCTCAGCT CAACACAGT CTGACACCAG AGCCCACTTC CATCCTCTCT GGTGTGAGGC ACAGATCGA GTTGGTGTC AACTGTGGTC TCGGGTGAAG GTAGGAGAGA CCACACTCCG
1441	ACAGCGAGG CAGCATCTGG AGGAGCTCTG CAGCCTCCAC ACCTACCAG ACCTCCCAGG TGTCGCTCCC GTCGTAGACC TCCTCGAGAC GTCGGAGGTG TGGATGTTGC TGGAGGGTCC
1501	GCTGGGCTCA GGAAAACAA GCCACTGCTT TACAGGACAG GGGTTGAAG CTGAGCCCCC CGACCCGAGT CCTTTTGGT CGGTGACGAA ATGTCTGTG CCCCAACTTC GACTCGGGC
1561	CCTCACACCC ACCCCCATGC ACTCAAAGAT TGGATTTTAC AGCTACTTGC AATCAAAAT GGAGTGTGG TGGGGGTACG TGAGTTTCTA ACCTAAATG TCGATGAACG TTAAGTTTA

MATCH WITH FIG. 5D

7/12

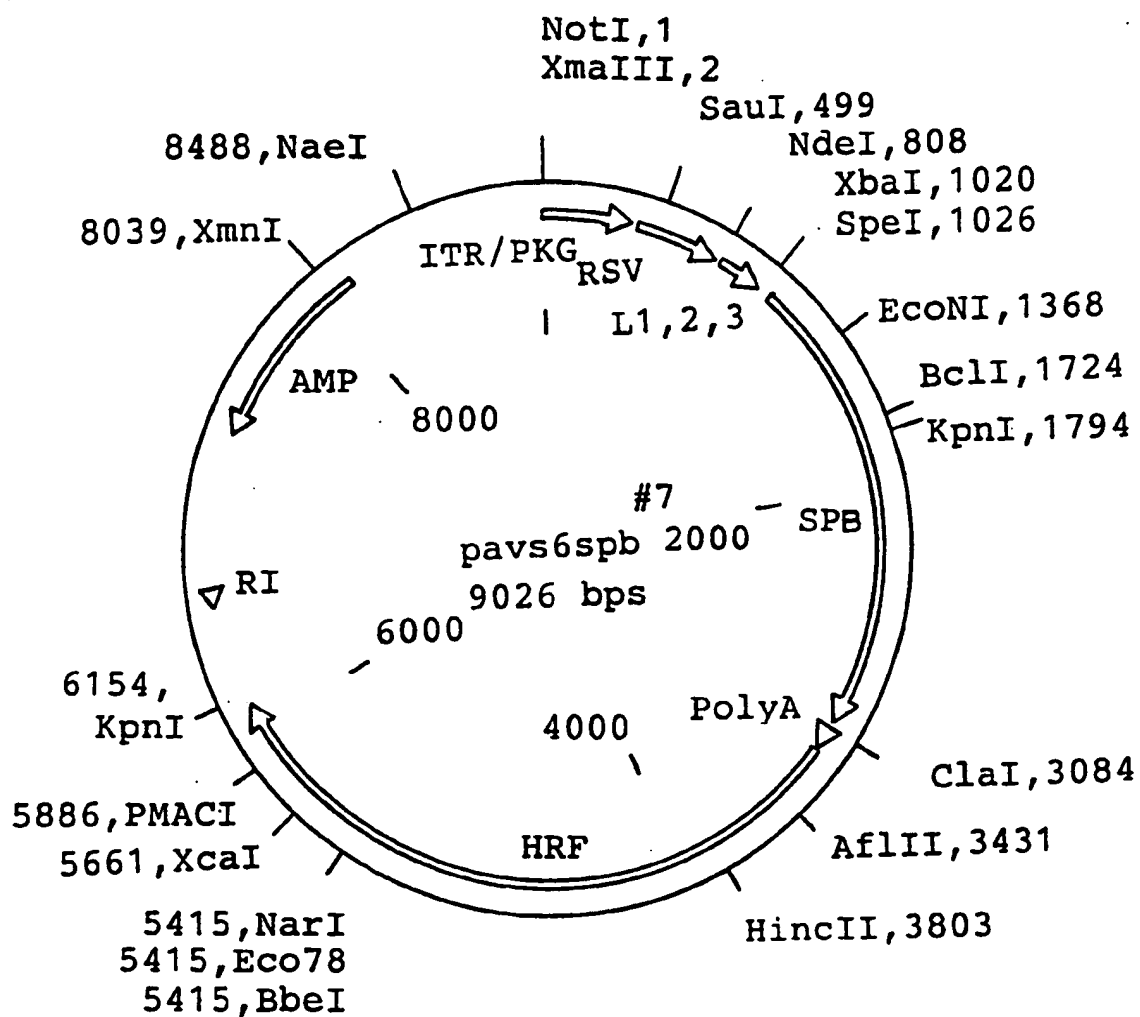
MATCH WITH FIG. 5C

1621	TCAGAAGAAAT	AAAAAATGGG	AACATACAGA	ACTCTAAAAG	ATAGACATCA	GAAATTGTTA
	AGTCTTCTTA	TTTTTTACCC	TTGTATGTCT	TGAGATTTTC	TATCTGTAGT	CTTTAAACAAT
1681	AGTTAAGCTT	TTTCAAAAAA	TCAGCAATTTC	CCAGCGTAGT	CAAGGGTGGA	CATGCACGCG
	TCAATTTCGAA	AAAGTTTITT	AGTCGTTAAG	GGTCGCATCA	GTTCCCCACCT	GTACGTGCGC
1741	TCTGGCATGA	TGGGATGGCG	ACCGGGCAAG	CTTCTCTTCC	CGAGATCGTC	TGCTGCTTGA
	AGACCGTACT	ACCCTACCGC	TGGCCCCGTT	GAAAGAAAGGA	GCTCTAGCAG	ACGACGAACT
1801	GAGCTATTGC	TTTGTTAAGA	TATAAAAAGG	GGTTTCTTTT	TGCTCTTCTG	TAAGGTGGAC
	CTCGATAACG	AAACAATTCT	ATATTTTCC	CCAAAGAAAA	ACAGAAAGAC	ATTCCACCTG
1861	TTCCAGCTTT	TGATTGAAAG	TCCTAGGGTG	ATTCTATTTC	TGCTGTGATT	TATCTGCTGA
	AAGGTCGAAA	ACTAACTTTC	AGGATCCCCAC	TAAGATAAAG	ACGACACTAA	ATAGACGACT
1921	AAGTCTAGCT	GGGGTTGTGC	AAGCTAGGGA	CCCATTCCTA	TGTAATACAA	TGTCTGCACC
	TTCGAGTCGA	CCCCAACACG	TTCGATCCCT	GGGTAAGGAT	ACATTATGTT	ACAGACGTGG
1981	AATGCTAATA	AAGTCCCTATT	CTCTTTTATC	GGAATT		
	TTACGATTAT	TTCAGGATAA	GAGAAAAATAG	CCTTAA		

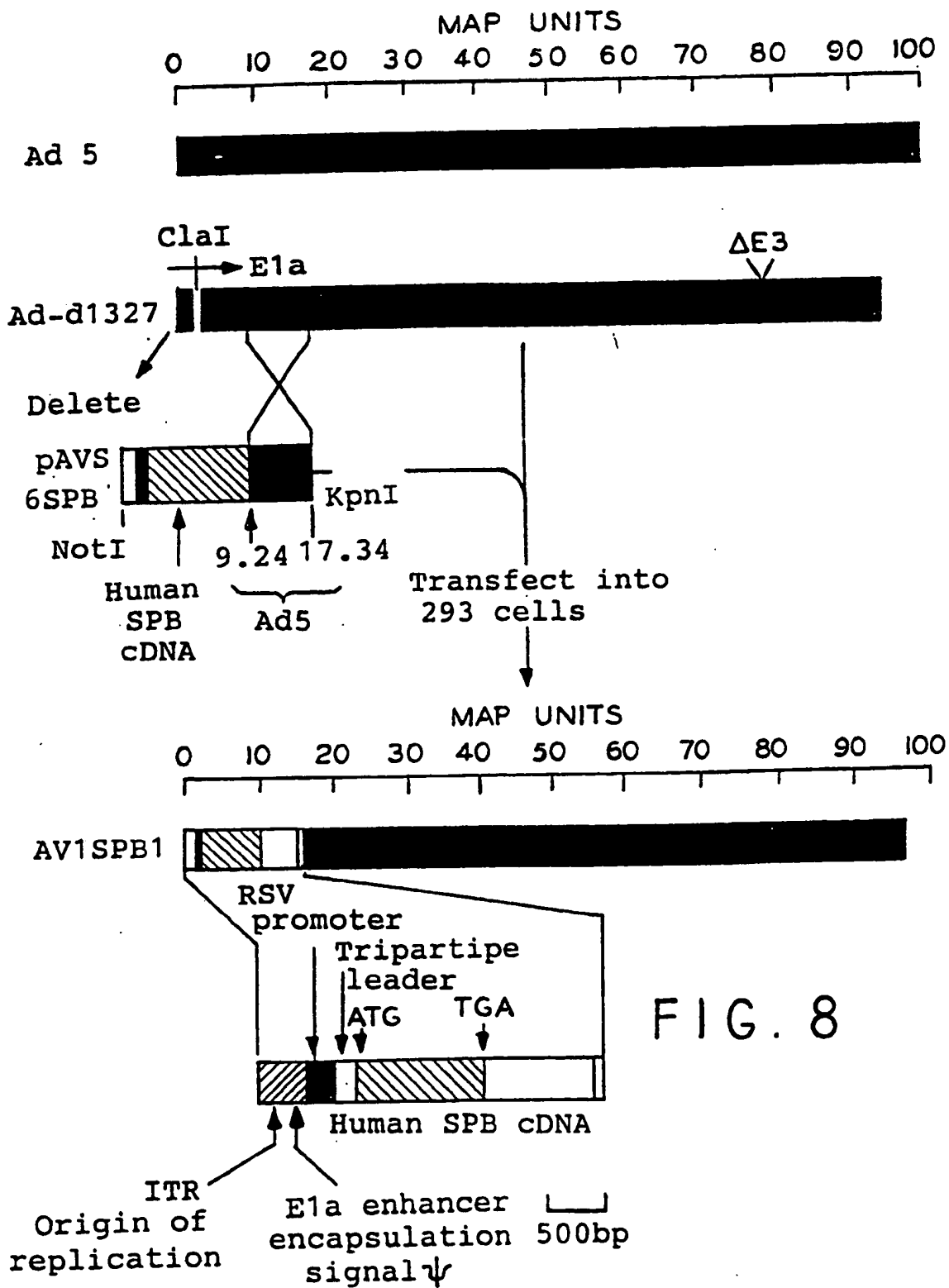
FIG. 5D

8/12

FIG. 7



9/12



10/12

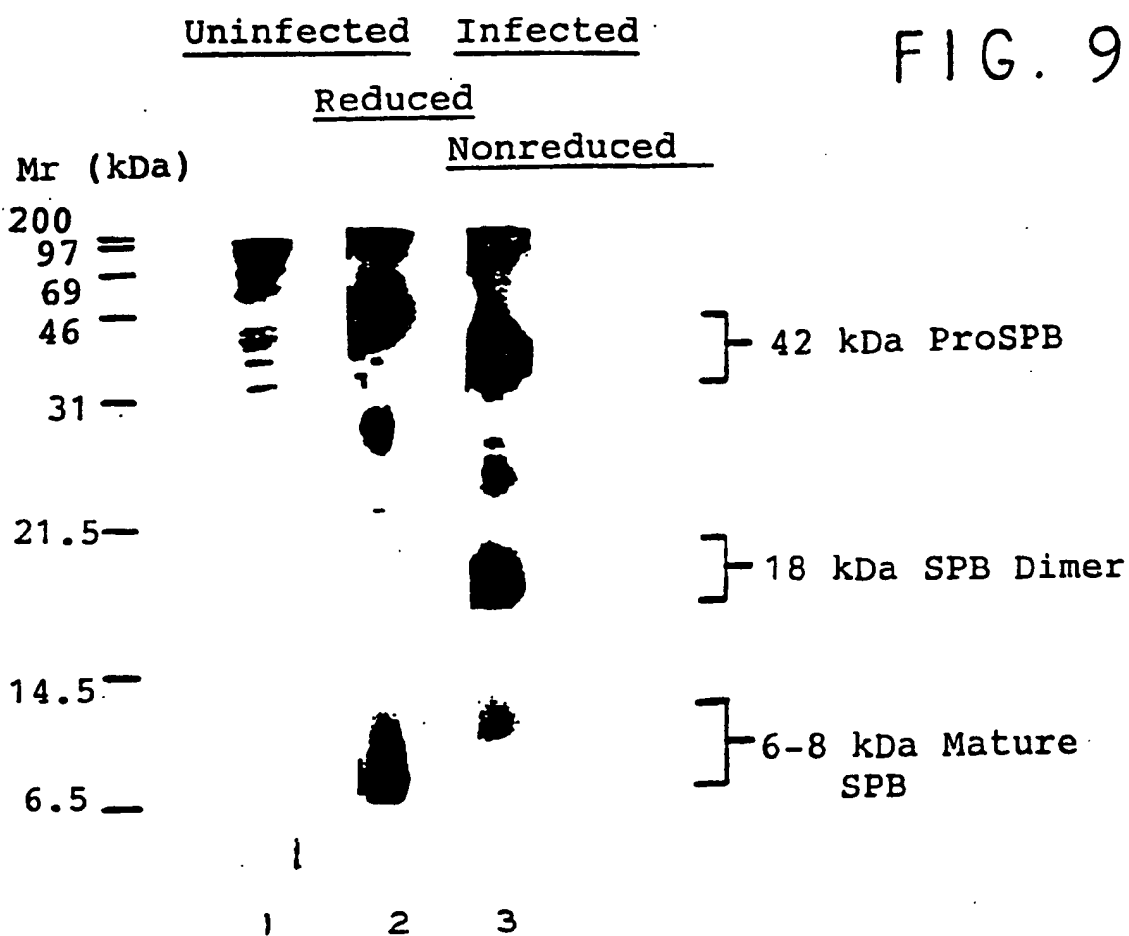
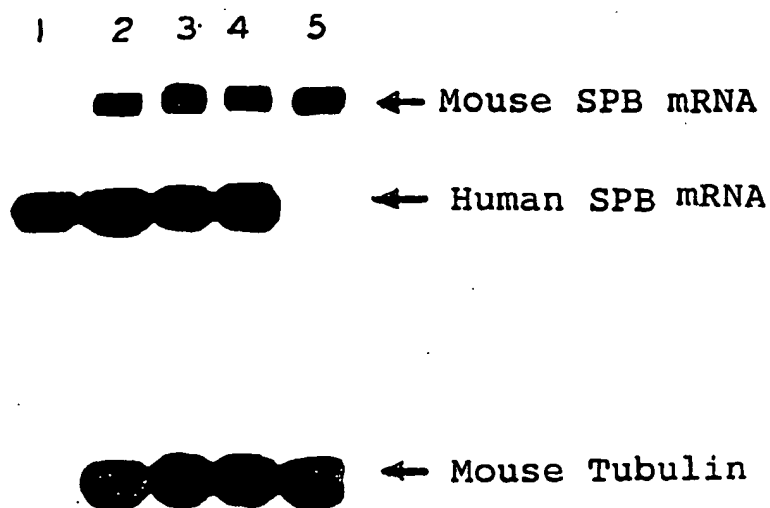
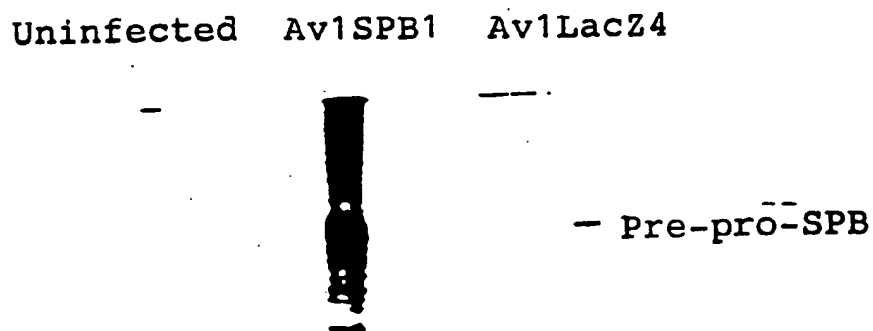
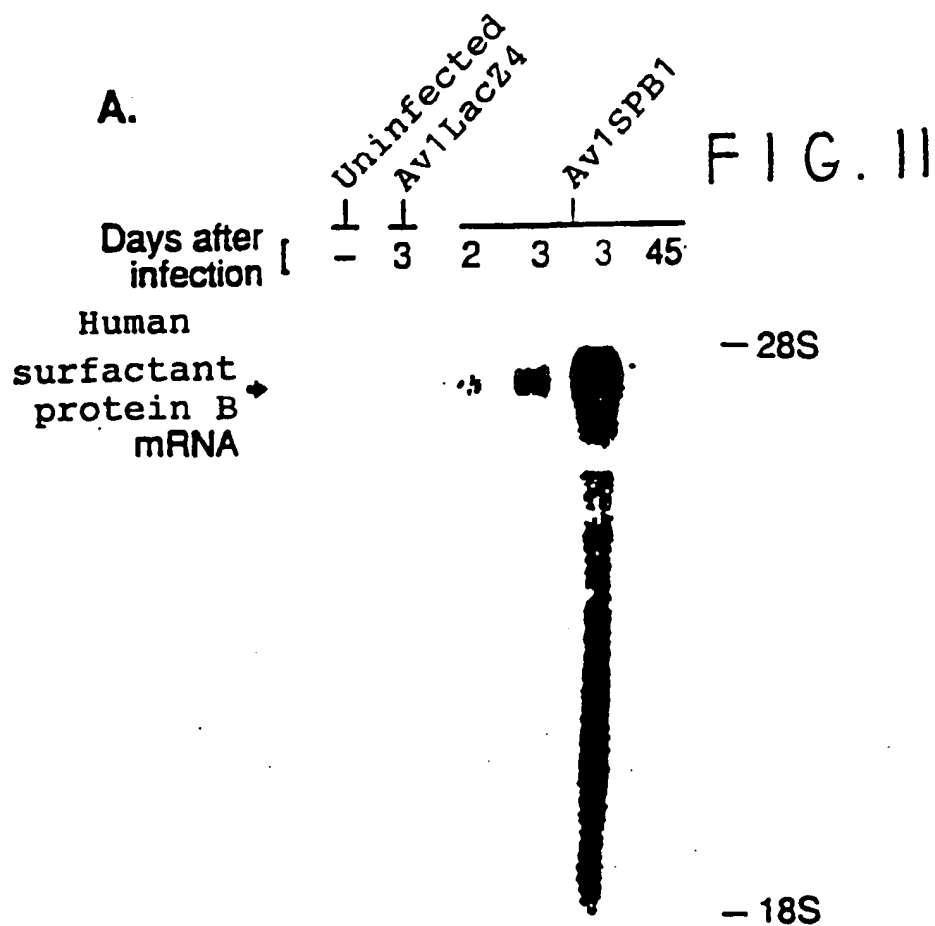


FIG. 10



SUBSTITUTE SHEET (RULE 26)

11/12



12/12

FIG. 14

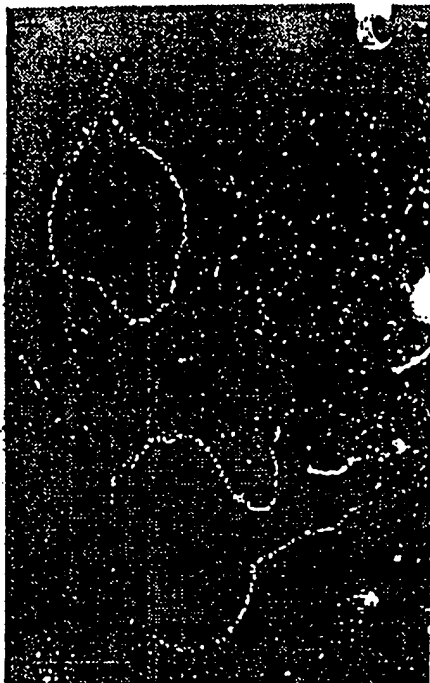


FIG. 16

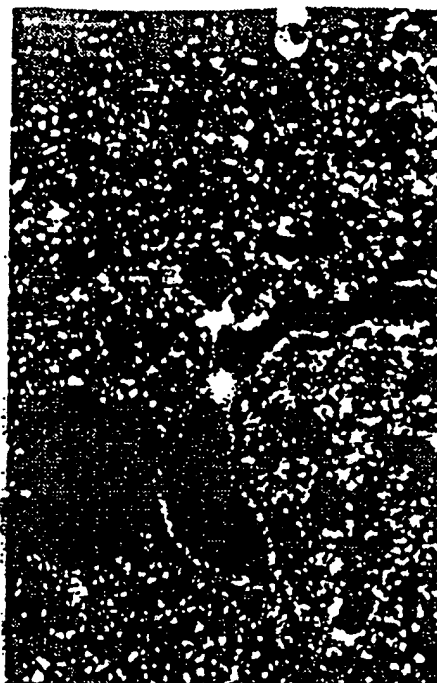
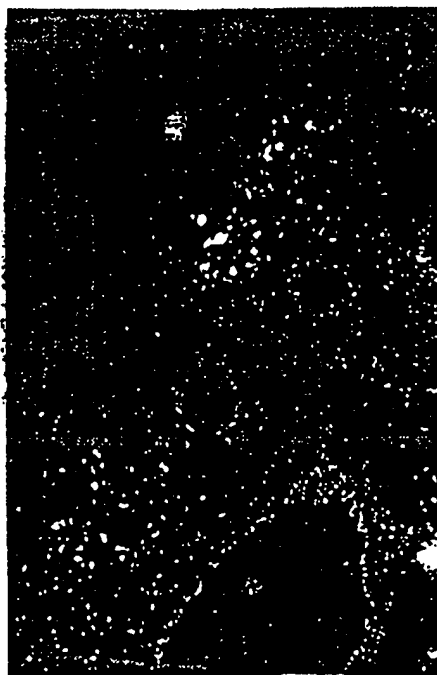


FIG. 13



FIG. 15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03831

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A01N 63/00; A61K 37/00; C07H 17/00; C12N 5/00, 7/00, 15/00

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93A, 93R; 435/172.1, 172.3, 235.1, 240.2, 320.1; 536/23.1, 23.5, 24.1, 24.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	W. Doerfler, "Adenovirus DNA", published 1986, by Martinus Nijhoff Publishing (Boston), pages 53-95, see the entire document.	1-24
Y	Cell, Volume 68, issued 10 January 1992, M.A. Rosenfeld et al, "In Vivo Transfer of the Human Cystic Fibrosis Transmembrane Conductance Regulator Gene to the Airway Epithelium", pages 143-155, see the entire document.	1-24
Y	Science, Volume 252, issued 19 April 1991, M. A. Rosenfeld et al, "Adenovirus-Mediated Transfer of a Recombinant α 1-Antitrypsin Gene to the Lung Epithelium in Vivo", pages 431-434, see the entire document.	1-24

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A document defining the general state of the art which is not considered to be part of particular relevance	* X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E earlier document published on or after the international filing date	* Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* Z document member of the same patent family
* O document referring to an oral disclosure, use, exhibition or other means	
* P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 MAY 1994

Date of mailing of the international search report

JUN 22 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

BRUCE CAMPBELL

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03831

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DNA, Volume 8, No. 2, issued 1989, T.J. Pilot-Matias et al, "Structure and Organization of the Gene Encoding Human Pulmonary Surfactant Proteolipid SP-B", pages 75-86, see the entire document.	1-24
Y	Journal of Clinical Investigation, Volume 81, issued March 1988, S.D. Revak et al, "Use of Human Surfactant Low Molecular Weight Apoproteins in the Reconstitution of Surfactant Biologic Activity", pages 826-833, see the entire document.	15-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03831

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/93A, 93R; 435/172.1, 172.3, 235.1, 240.2, 320.1; 536/23.1, 23.5, 24.1, 24.2

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, EXCERPTA MEDICA, DERWENT BIOTECHNOLOGY ABSTRACTS, CHEMICAL ABSTRACTS
search terms: surfactant and (pulmonary or lung), adenovirus vector?, gene therapy

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.